International Journal of Bio-Technology
and Research (IJBTR)
ISSN(P): 2249-6858; ISSN(E): 2249-796X
Vol. 6, Issue 1, Feb 2016, 43-48

PREBIOTICS FORTIFIED WITH FRUIT JUICES-A GOOD CARRIER FOR PROBIOTICS

MRADULA GUPTA & SOMESH SHARMA

School of Bioengineering and Food Technology, Shoolini University of Biotechnology and Management Sciences, Solan, Himachal Pradesh, India

ABSTRACT

The need of the hour is to develop a beverage with health benefits beyond their nutritional value. In wider context beverages that are, or are perceived to be, "better for you", i.e. functional and fortified. The study was conducted to develop a probiotic millet milk beverage fortified with different blends of pineapple juice using following ratios (Millet milk: Pineapple Juice) 90:10, 80:20, 70:30, 60:40, 50:50. Proximate composition revealed that the moisture content in the end product was 90.6± 0.3% to 98.2 ± 0.3 % whereas protein ranged from 1.617 ± 0.001 % to 1.365 ± 0.001 %, fat 0.077 ± 0.001 % to 0.278 ± 0.01 %, crude fiber 1.06 ± 0.001 % to 1.00, ash content 0.268 ± 0.005 % to 0.465 ± 0.001 % and non reducing sugar 0.48±0.10 % to 0.9±0.05% in different blends. The pH ranges from3.55±0.3 to 4.06±0.4 and total viable count 1.04x10^{8}cfu/ml to 2.78x10^8 among all blends. The blends T3,T4 and T5 were selected as the most preferred treatments among all blends based on nutritional as well as sensory point of view and could be stored at a minimum period of 30 days at 4°C, without any significant changes in quality. Summarily, results suggested that potentially symbiotic beverage presents suitable physicochemical parameters and supports the growth of LAB as it contains cell viability above the minimum recommended for a probiotic product (6 Log10 cfu/ml based on a daily dose of 100 ml).

KEYWORDS: Functional Beverage, Millet Milk, Probiotic Beverages, Product Development, L. plantarum

Received: Dec 18, 2015**; Accepted:** Jan 08, 2016**; Published:** Feb 09, 2016; **Paper Id.:** IJBTRFEB20165

Original Article

INTRODUCTION

Foods are considered healthy and functional when they exert specific body functions, in addition to the nutritional effects. Dietary fibers, oligosaccharides and probiotics that are helpful in maintaining the balance of intestine are few well known examples of functional foods. In addition to these, vitamins, minerals, lipids, and phytonutrients are also functional ingredients. The requirement of functional foods is growing day by day because of increased awareness about the usefulness of these foods on health among consumers (Tripathi and Giri, 2014). Probiotic foods comprise between 60-70% of the total functional food market (Perricone et al., 2015). Generally lactic acid bacteria are incorporated in probiotic foods on a regular basis (Reid, 2008). Consumers are increasingly demanding these foods as these are health enhancing products and also are proving a new era and need for new non dairy substrates for other probiotic product development. According to previous researches, it have been reported that fruit and vegetable juices may be next substrates as they contain nutrients easily assimilated by probiotics, to be used as carriers of probiotic bacteria. It has been also reported that oat, barley and malt substrates can support the growth of single and mix-culture fermentations of probiotic microorganisms and in this regard they can enhance lactobacilli tolerance to the harsh conditions of the gastrointestinal tract (Salmerón et al., 2015). Additionally, to the

proven potential of cereal substrates to support the growth of probiotic microorganisms these have been associated with the risk reduction of chronic diseases such as obesity, cardiovascular disease, type 2 diabetes, and some cancers (Wang et al., 2014). Accordingly the use of cereal substrates has a great potential to develop novel functional beverages that promote the gastrointestinal health and can prevent chronic diseases. If cereals fulfill the probiotic requirements and have acceptable physicochemical characteristics and organoleptic properties as well, then could be used for cereal based fermented beverages. Food technology encounters several challenges during the development of novel products. Consumer sensory evaluation is used to find out whether a consumer likes a product, prefers it over another, or finds it acceptable based on its sensory characteristics (Earle et al., 2001).So, now a days, fruit juices, vegetables and cereals constitute an emerging segment of nonconventional dairy substrates for the design of probiotic beverages that will require sensory and physicochemical characterization for quality control and further product development(Chattopadhyay et al., 2013; Pereira et al., 2013; Perricone et al., 2014). The main objective of this work was also to develop a synbiotic beverage (probiotic and prebiotic) which was further fortified with different ratios of pineapple juices and analyzed for the viability of probiotic bacteria over time.

MATERIALS AND METHODS

Procurement of Raw Material

Pearl millet grains (*Pennisetum gluacum*) were procured from Department of Agronomy, H.A.U., Hisar.

Pineapple fruit (*Ananas comosus L.*) purchased at local markets (Solan) was used freshly after extracting its juice.

Microorganisms and Inocula Preparation

For the inocula preparation isolated *Lactobacillus plantarum* was added directly to sterilized MRS broth and incubated at 37°C for 24 h. The strain *Lactobacillus plantarum* was isolated from the fermented fig pickle earlier and was further identified by 16S rRNA sequencing and was stored at -30 °C in Man Rogosa Sharpe (MRS) broth containing 10% (v/v) of glycerol. Stocks were prepared routinely in MRS medium. Before use, the lactobacilli cultures were propagated in sterile millet milk (starch-free extracts of flour– water suspensions prepared from millet) at 37 °C for 20 h.

Physicochemical Analysis

The moisture, protein (micro-kjeldahl method), fat, crude fiber and ash contents and carbohydrate analyses were performed according to (AOAC, 2000).

Physical Analysis

The pH was determined by digital pH meter calibrated with standard buffer solutions. Titratable acidity was determined as lactic acid percentage by titrating with 0.1 NaOH, using phenolphthalein as an indicator (Kurt et al., 1996).

Development of the Functional Beverages

The millet milk used as substrate was mainly starch-free extracts of millet flour and water suspension prepared from common pearl millet (from a local producer). The common pearl millet was processed with steeping in water at 20°C for 36 hrs followed by germination at 10°C for 24 h and 18°C for 72 h and finally drying at 34°C. The grains were then grounded using laboratory grinder. The pearl millet flour was mixed with 5% (w/w) sterilized boiled water and heated at 90 ± 5°C for one hour and then cooled down at 50 ± 5°C for 30 min. The starch-free millet milk was further sterilized at 121°C for 20 min. Further, the prepared millet milk was mixed with pineapple juice in different ratios. The experimental

design is given in Figure 1. The blended mixture was then inoculated with 1% *L.plantarum* and observations for various quality attributes were recorded. Samples were aseptically taken to perform chemical and physiochemical analysis.

Figure 1: Development and fortification of probiotic millet milk beverage

Millet
↓
Steeping at 25°C for 24 h
↓
Germination for 30°C for 48 h
↓
Kilning at 35°C
↓
Cleaning of dried roots and shoots
↓
Milling
↓
Addition of water (50:50)
↓
Boiling for 30 minutes at 80°C
↓
Adding more water to make millet milk
↓
Cooling and settling for 30 min
↓
Sterilization at 121°C for 15 min
↓
Inoculation volume (1%)
↓
Blending of millet milk with pine apple juice

90:10

80:20

70:30

60:40

50:50
↓
Bottling and Storage

Figure 1: Development and Fortification of Probiotic Millet Milk Beverage

Microbiological Analysis

The total number of viable bacteria in sample were enumerated by the standard plate count method using plate count agar as described by (Houghtby et al.,1993).Triplicate plates were incubated at 37^{o}C for 24-48 h. The colonies were counted and expressed as log cfu/ml of the product.

Sensory Evaluation

The five beverage samples were scored for color, taste, texture, flavor and overall acceptability.

Stability Study

To evaluate the stability of the beverage, the most accepted blend was stored at refrigerated conditions (4°C) subjected to physicochemical, microbiological and sensory acceptance tests on days 0, 10, 20 and 30 of storage. Sample was also analyzed for molds and yeasts. The total coliform counts were by standard plate count method (Houghtby et al.,1993).

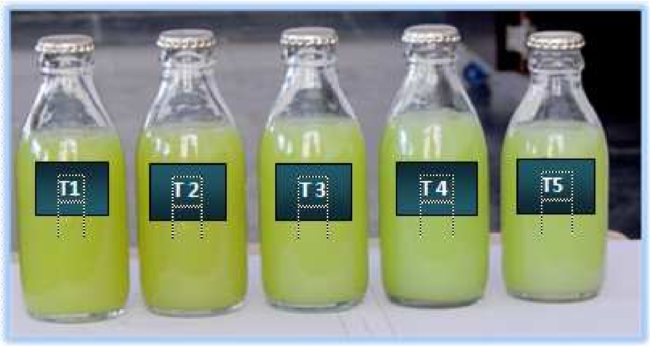
RESULTS

Table 1: Proximate Composition of Probiotic Millet Milk among All Blends (100ml)

	Moisture (%)	Protein (%)	Fat (%)	Crude Fiber (%)	Ash Content (%)	Non Reducing Sugar (%)
T1	98.2±0.3	1.617±0.001	0.077±0.001	1.06±0.001	0.268±0.005	0.90±0.05
T2	91.7±0.5	1.554±0.001	0.127±0.001	1.06±0.010	0.318±0.001	0.63±0.07
T3	90.6±0.3	1.491±0.001	0.177±0.001	1.03±0.001	0.368±0.001	0.51±0.09
T4	95.7±0.7	1.428±0.001	0.228±0.001	1.02±0.011	0.416±0.000	0.49±0.04
T5	93.6±0.2	1.365±0.001	0.278±0.001	1.01±0.000	0.465±0.001	0.48±0.10

(Millet milk: Pineapple juice) T1=90:10, T2= 80:20, T3= 70:30, T4= 60:40, T5= 50:50

Moisture content ranged between 90.6± 0.3% to 98.2 ± 0.3 %.Moisture in T1 was high in comparison to other treatments, followed by T4, T5, T2 and lowest in T3. Protein content ranged between 1.617 ± 0.001 % to 1.365 ± 0.001 %. Protein in T1 was highest followed by T2, T3, T4 and T5 was lowest. Fat ranged between 0.077 ± 0.001 % to 0.278 ± 0.01 %, Crude fiber ranged between 1.06 ± 0.001 % to 1.00± 0.01 % whereas ash ranged between 0.268 ± 0.005 % to 0.465 ± 0.001 %. Ash content was high in T5 as compared to others and lowest in T1. Non Reducing Sugar were found maximum in blend T1 followed by T2, T3, T4 and T5.



Picture 1: Probiotic Pineapple Millet Milk Beverage Blends T1,T2,T3,T4,T5.

Table 2: Physico-Chemical Parameters of Probiotic Millet Milk among All Blends

Blends	Titrable acidity	pH	Total Viable Count (CFU/Ml)
T1	0.61±0.04	3.55±0.3	2.78×10^8
T2	0.51±0.02	3.81±0.8	2.01×10^8
T3	0.45±0.06	3.59±0.2	1.04×10^8
T4	0.49±0.05	3.97±0.3	1.81×10^8
T5	0.62±0.06	4.06±0.4	1.56×10^8

Beverages pH ranged from 3.55 ± 0.03 to 4.06 ± 0.4 as shown in table 2 which is an acidic beverage. Titratable acidity of beverage as lactic acid was found to range from 0.45% to 0.62%. Among all blends, the blends T3,T4 and T5 presented good acceptance by consumers. This result is also important from the nutritional point of view.

DISCUSSIONS

Pearl millet grain undergoes malting before milling and is used in beverage making. The malting process enables the production of enzymes such as amylases, which hydrolyze grain starch to sugar (glucose and maltose) (Taylor, 2004) and thus free sugar is available for lactic acid bacteria to ferment. The lactic acid bacteria especially lactobacillus ferments the sugar (glucose) into lactic acid and yeast into ethanol and carbon dioxide (Dewar and Taylor, 1999). Culture was used as inocula at 1% (v/v) in each millet milk during the preparation of the Probiotic millet milk beverages fortified with fruit juices to get the cell viability above the minimum recommended for a probiotic product (6 Log10 cfu/ml based on a daily dose of 100 ml) (Rathore et al., 2012).

Beverage is very high in moisture content, which is above 90 %. High water content will provide consumers with water intake through beverage and this will helps in keeping them hydrated. Ash is a good indicator of minerals. Beverage has a considerable amount of proteins. It has insoluble fiber, which can be contributed from the ingredients as they come from plant materials. The differences in the chemical properties of millet milk blends could be due to the different chemical composition of the original cereal pastes and cereal blends. The acidic pH inhibits and favors certain microorganism in beverage. It has been reported that at a pH below 3.8 the growth of most food spoilage bacteria can be inhibited (Battey and Schaffner, 2001). Lactobacilli are generally resistant and survive in juices with pH ranging from 3.7 to 4.3 (Tripathi et al., 2014). According to (Cruz et al., 2010), the ideal count of probiotics recommended for fermented dairy beverages is between 10^6 and 10^7 viable cells/ml. (Molin et al., 2001) reported that to make the fermented cereal gruel more acceptable to consumers in the industrialized world, it mixed into a fruit drink.

CONCLUSIONS

This study shows a new possibility to make an acceptable fermented product based mainly on millet which are suitable substrates that's can support high cell viability for different probiotic strains. It is a good source of 'fibers that contribute to the balance of the intestinal microflora and had the potential to present prebiotic effects including proteins. Fruit juices when combined with prebiotics can represent a suitable carrier for probiotics. In this manner, this symbiotic product (probiotics and prebiotics) can come under nutraceutical product, designed for a wide range of consumers. In India where most of the drinks are lactose based, these beverages are boon for lactose intolerant people. Beverage is characterized by lactic acid bacteria that have important function.

There are some challenges to overcome, *i.e.*, the survival of probiotics, and the effects on the sensory traits, post acidification however, there are some possible solutions that show that there is a promising way.

REFERANCES

1. *AOAC International. (2005). Official methods of analysis of AOAC International. AOAC International.*
2. *Battey, A. S., Duffy, S., & Schaffner, D. W. (2001). Modelling mould spoilage in cold-filled ready-to-drink beverages by Aspergillus niger and Penicillium spinulosum. Food Microbiology, 18(5), 521-529.*
3. *Champagne, C. P., & Gardner, N. J. (2008). Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses.Food Research International, 41(5), 539-543.*
4. *Chattopadhyay, S., Raychaudhuri, U., & Chakraborty, R. (2013). Optimization of soy dessert on sensory, color, and rheological parameters using response surface methodology. Food Science and Biotechnology,22(1), 47-54.*

5. *Cruz, A. G., Faria, J. A. F., Walter, E. H. M., Andrade, R. R., Cavalcanti, R. N., Oliveira, C. A. F., & Granato, D. (2010). Processing optimization of probiotic yogurt containing glucose oxidase using response surface methodology. Journal of Dairy Science, 93(11), 5059-5068.*

6. *Dewar, J., & Taylor, J.R.N. (1999). Beverages from sorghum and millet. In: Encyclopaedia of Food Microbiology. Robinson, R.K., Batt, C.A., & Patel, P.D (Eds). Academic Press. 3, pp 759-767.*

7. *Earle, R., & Anderson, A. (Eds.). (2001). Food Product Development: Maximizing Success. CRC Press.*

8. *Houghtby, G. A., Maturin, L. J., & Koenig, E. K. (1992). Microbiological count methods. Standard Methods for the Analysis of Dairy Products. 15th ed.–TR Marshall, ed. Am. Publ. Health Assoc., Inc., Washington, DC, 213-246.*

9. *Kurt, A., Cakmakci, S., & Caglar, A. (1996). A guide book of analysis methods of milk and milk products. Erzurum, Turkey: Atatürk University, Agricultural Faculty Pub. no18.*

10. *Molin, G. (2001). Probiotics in foods not containing milk or milk constituents, with special reference to Lactobacillus plantarum 299v. The American journal of clinical nutrition, 73(2), 380s-385s.*

11. *Pereira, A. L. F., Maciel, T. C., & Rodrigues, S. (2011). Probiotic beverage from cashew apple juice fermented with Lactobacillus casei. Food Research International, 44(5), 1276-1283.*

12. *Perricone, M., Bevilacqua, A., Altieri, C., Sinigaglia, M., & Corbo, M. R. (2015). Challenges for the Production of Probiotic Fruit Juices. Beverages,1(2), 95-103.*

13. *Rathore, S., Salmerón, I., & Pandiella, S. S. (2012). Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. Food microbiology, 30(1), 239-244.*

14. *Reid, G. (2008). Probiotics and prebiotics–progress and challenges. International Dairy Journal, 18(10), 969-975.*

15. *Salmerón, I., Thomas, K., & Pandiella, S. S. (2015). Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. Journal of Functional Foods, 15, 106-115.*

16. *Taylor, J.R.N. (2004). Millet. In: Encyclopedia of Grain Science. Wrigley, C.W., Corke, H., & Walkers, C. (Eds). 2, 253-261.*

17. *Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. Journal of functional foods, 9, 225-241.*

18. *Wang, T., He, F., & Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. Journal of Functional Foods, 7, 101–111.*